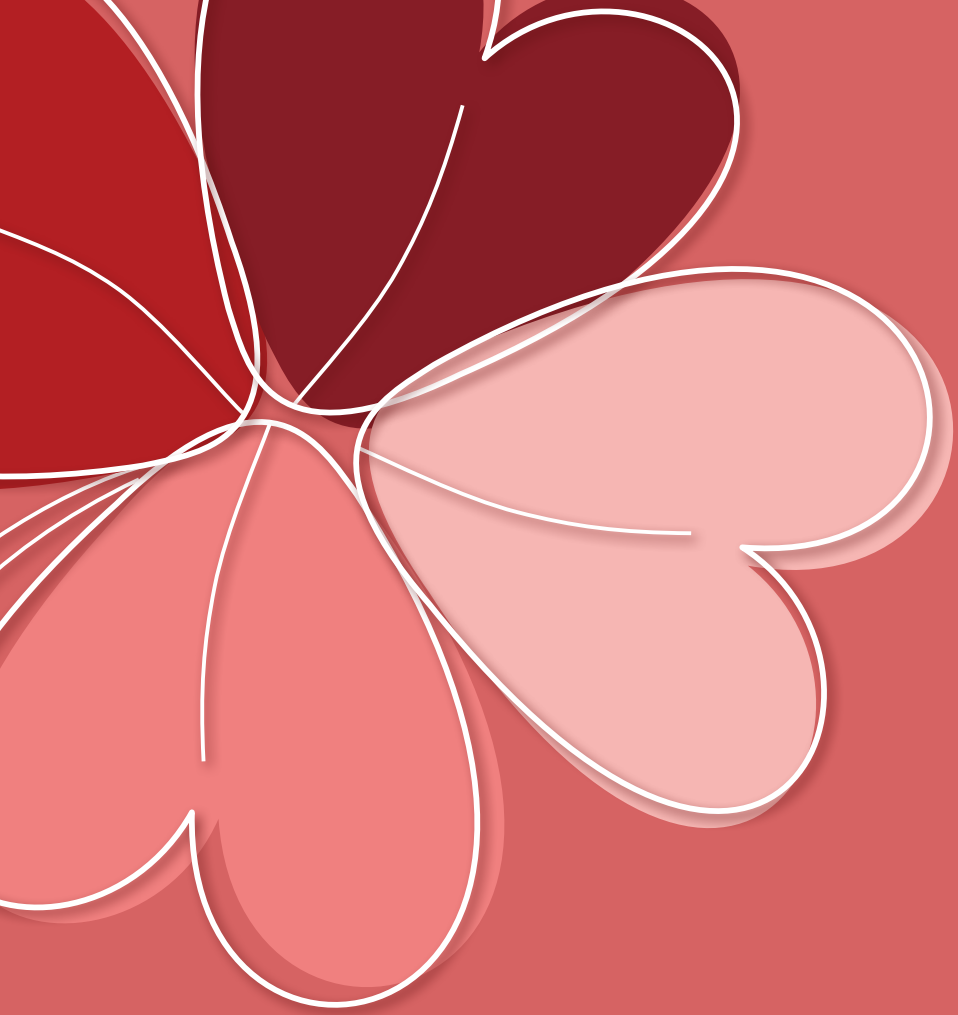
Protein intake and diet quality in old age and their role in nutritional and functional status

Hengeveld, L.M.

2021

document version
Publisher's PDF, also known as Version of record



CHAPTER 6

Prospective associations of protein intake parameters with muscle strength and physical performance in community-dwelling older men and women from the Quebec NuAge cohort

Linda M. Hengeveld, Stéphanie Chevalier, Marjolein Visser,
Pierrette Gaudreau, Nancy Presse

Revision accepted for publication in the American Journal of Clinical Nutrition

ABSTRACT

Background: Dietary protein has been related to muscle function in ageing. Beyond total intake, parameters such as protein distribution across meals might also be important.

Objective: To examine prospective associations of different protein intake parameters with muscle strength and physical performance in community-dwelling older men and women.

Design: 524 men and 574 women aged 67 to 84 years at baseline (T1) were followed annually for three years (T2, T3, T4). Outcomes included handgrip strength (kPa), knee extensor strength (kg) and physical performance (Timed Up and Go (s)) at T4, and their 3-year changes (T4 minus T1). Protein intake parameters were assessed using nine 24-hour recalls collected over three years (T1, T2, T3) and included: daily total intake (g/d), number of protein-providing meals and snacks, and protein distribution across meals (expressed as coefficient of variation). Associations were examined by multivariable linear regression models including all protein intake parameters simultaneously. Also, the optimal protein dose (g) per meal for maximum effect size of total daily intake was determined.

Results: Higher daily protein intake was associated with better knee extensor strength and physical performance at T4 in both sexes and less physical performance decline in women. Optimal protein doses per meal were 30-35 g in men and 35-50 g in women for these outcomes. In men, more uneven protein distribution was associated with better physical performance at T4 and less handgrip strength decline. In women, higher number of protein-providing snacks was associated with better handgrip strength and knee extensor strength at T4 and less handgrip strength decline. In neither sex was number of protein-providing meals associated with outcomes.

Conclusions: Higher daily protein intake, up to 30-50 g protein/meal, may contribute to better knee extensor strength and physical performance in generally well-functioning older men and women. The role of protein distribution across meals and number of protein-providing meals and snacks remains uncertain.

INTRODUCTION

Muscle strength diminishes with age [1] and contributes to decreased physical performance [2] and higher risks of frailty, disability and mortality in older adults [3, 4]. Dietary protein intake is a key modifiable factor affecting muscle metabolism [3-5]. Nonetheless, there is an ongoing debate on protein recommendations for older people, which currently vary from 0.8 to 1.2 g/kg body weight (BW)/d [6-8]. Protein recommendations may be difficult to establish because beyond the daily quantity, other protein intake parameters have been evoked as being potentially involved in the anabolic response to protein [6, 9]. These include notably the evenness of protein intake distribution across meals [6, 10, 11] and an optimal protein dose per meal of 25 to 35 g beyond which muscle protein synthesis (MPS) is no further increased in older adults [6, 10, 11]. Studies also indicated that more frequent consumption of protein-providing meals may be related to better muscle strength [11, 12]. However, such protein intake parameters may not be independent from one another. Reaching 30 g of protein per meal three times/day probably leads to an even protein intake distribution across meals and a relative high daily protein intake [13-16]. Furthermore, protein requirements may differ between men and women due to sex-differences in body composition [17], hormonal milieu [17, 18], MPS rate [19, 20] and sensitivity to anabolic stimuli [18, 20]. Insight into the independent role of each protein intake parameter in muscle strength and physical performance of older men and women is required to optimise protein recommendations.

Observational studies are most appropriate to investigate how protein intake parameters relate to clinically relevant outcomes such as muscle strength and physical performance. However, most studies focused only on daily protein intake – many of them being limited by the use of a food frequency questionnaire, which cannot estimate protein intake per eating occasion. Moreover, only four studies presented prospective data. One prospective study showed that higher protein intake was associated with less decline in handgrip strength in both sexes [21]. Others observed no association with change in handgrip strength [22, 23], knee extensor strength [24] or physical performance [23]. Only two prospective studies examined other parameters of protein intake, specifically its distribution across meals. One showed that a more even protein intake distribution across meals was associated with higher handgrip strength throughout a 3-year follow-up in the NuAge cohort, but not with knee extensor strength or physical performance [25]. The other observed no associations with change in handgrip strength or physical

performance [23]. Among the factors that may explain this discrepancy, is the fact that not all protein intake parameters were examined simultaneously in one model.

A better understanding of how protein intake parameters can contribute to maintenance of muscle strength and physical performance requires studies having access to detailed dietary data and considering multiple protein intake parameters simultaneously while avoiding collinearity. Hence, the aim of the present study was to examine prospective associations of different protein intake parameters with muscle strength and physical performance after three years of follow-up, and with their 3-year change, in community-dwelling older men and women.

METHODS

Study population

Data used in the present study were from the NuAge Database and Biobank, containing detailed information on 1754 participants of the NuAge study (Quebec Longitudinal Study on Nutrition and Successful Aging). A complete description of the NuAge study can be found elsewhere [26]. In short, the NuAge cohort consists of generally healthy Quebec community-dwelling older men and women aged 67 to 84 years at baseline (2003-2005; T1). Inclusion criteria were, among others, able to walk without help, able to walk 100 metres or climb ten stairs without rest, free of disabilities in activities of daily living and not cognitively impaired (Modified Mini-Mental State Examination (3MS) score >79). People suffering from severe health conditions were excluded [26]. Baseline measurements (T1) took place at the Research Centers of either the Montreal or Sherbrooke Geriatric University Institutes. Follow-up data were retrieved annually for three subsequent years (T2, T3 and T4). At each clinic visit, nutritional, functional, medical and social variables were assessed by trained research dietitians and nurses using computer-assisted personal interview software. Dietary intake was assessed annually with three non-consecutive 24-hour dietary recalls. The NuAge Database and Biobank as well as the present study have been approved by the Research Ethics Board of the Centre intégré universitaire de santé et de services sociaux de l'Estrie - Centre hospitalier universitaire de Sherbrooke (CIUSSS de l'Estrie-CHUS).

Analytic sample

We aimed to examine associations of different protein intake parameters with (1) muscle strength and physical performance after three years of follow-up (i.e. at T4) and (2) the 3-year change in muscle strength and physical performance (i.e. T4 minus T1). To this end, we excluded participants with missing data on muscle

strength or physical performance at T1 (*n*=23) or T4 (*n*=473). Furthermore, we excluded participants with one or more missing 24-hour dietary recalls at T1, T2 or T3 to achieve the most precise estimate of each protein intake parameter (*n*=116) and then excluded those with missing data on covariates that were considered important confounders (*n*=44), leaving an analytic sample of 524 male and 574 female participants (**Supplemental Figure S1**).

Assessment of muscle strength and physical performance

Measures of muscle strength included handgrip strength and knee extensor strength, assessed according to a predefined standardised protocol in NuAge. Handgrip strength was assessed using a pneumatic dynamometer (Martin vigorimeter), which measures the force of compression (kPa). Participants were seated with the shoulder adducted and the elbow flexed at 90 degrees and were encouraged to squeeze the bulb at maximal force for a maximum of six seconds. Three maximum contractions were recorded at each hand, starting with the dominant side. Knee extensor strength was assessed using the dynamometer Microfet2 and the belt-resisted method [27], which measures the strength of the quadriceps (kg). Participants were seated with the knee flexed at 120 degrees and the foot on the ground, and encouraged to push at maximum force against the dynamometer placed in the centre of the distal third of their leg [27]. Three maximum contractions were recorded at each leg. For both muscle strength measures, the highest value of the six attempts at T4, regardless of the side, was used in our study. The 3-year changes in muscle strength were calculated as T4 minus T1, by using for T1 the highest value of the same side as T4 to ensure consistency. Physical performance was assessed using the Timed Up and Go (TUG) test, which measures the time (s) it takes to rise from a chair without using arms, walk three metres at usual pace, turn, return to the chair and sit down [28]. Participants were allowed to use walking aids but not assistance of the examiner. The test was completed once per participant after a practice trial. A higher value indicates more time to complete the TUG test, so poorer physical performance. The 3-year change in physical performance was calculated as T4 minus T1.

Dietary assessment

Three non-consecutive 24-hour dietary recalls (one face-to-face during the annual visit, two by telephone in the weeks following the visit) were collected on two randomly chosen weekdays and one weekend day. The 24-hour dietary recalls were administered by extensively trained registered dietitians. They followed the US Department of Agriculture (USDA) five-step multiple-pass method [26, 29] and used portion-size models and pictures of standardised food portions to increase

precision of the food estimates. Dietary intake data were obtained for seven eating occasions, including three meals (breakfast, lunch and dinner) and four snack moments (morning, afternoon, evening and night snack). Intakes of energy and nutrients were calculated using the CANDAT nutrient analysis program (version 10; Godin London), which was based on the 2007 Canadian Nutrient File. For the present study, we used all 24-hour dietary recalls collected prior measurement of outcomes at T4, so nine in total. Recalls from T4 were not used, because they were collected over the weeks following the measurement of outcomes at T4.

Composition of protein intake parameters

Five protein intake parameters were investigated: daily protein intake, number of protein-providing meals, number of protein-providing snacks, evenness of protein intake distribution across meals and the optimal protein dose per meal (i.e. the level of protein intake to be reached in each meal to maximise effect size on muscle strength and physical performance, assuming that an optimal dose actually exists). Except for the latter, all protein intake parameters were determined for each recall day separately and the mean of the nine days was used for analyses, as a reflection of the usual intake pattern.

Daily protein intake (g/d) comprised all protein consumed in one day. Protein-providing meals or snacks included those with >0 g protein. The mean number of protein-providing meals consumed per day was categorised into <2.86 meals/d, 2.86 to 2.99 meals/d and 3.00 meals/d. Number of protein-providing snacks was categorised into sex-specific tertiles. Evenness of protein intake distribution across meals was determined using the coefficient of variation (CV). For this, the standard deviation (SD) of the mean protein intake from meals (g/d) was divided by the mean protein intake from meals (g/d). A higher CV indicates less evenness of protein intake across the meals. Since previous studies have indicated that the number of protein-providing meals may be related to muscle strength [12, 30], we calculated the CV only over the number of protein-providing meals (which may differ per individual and per recall day) in order to obtain a CV that is independent of the number of meals with protein.

To investigate the optimal protein dose per meal, we recalculated the daily protein intake and the CV for each recall day of each participant, after having truncated the protein intake per eating occasion to a given maximal value (i.e. the potential optimal dose), presuming that any protein intake beyond this dose has no additional benefit to muscle strength or physical performance. Because of the uncertainty about the existence of an optimal dose per meal [31, 32], let alone the exact level of

this dose, we applied potential values varying from 15 to 70 g with 5-g increments. The highest value of 70 g corresponds to the 95th percentile of the distribution of protein intake per meal in our analytic sample. By this approach, we postulated that if an optimal protein dose per meal actually exists, daily protein intake and CV will be calculated with increasing error when truncating the protein dose per meal with values under or above the so-called optimal dose, translating into attenuated estimates of regression coefficients in statistical analyses.

Assessment of covariates

Sex, years of education, smoking status and number of prescribed medications were self-reported. Any alcohol consumption in the past three years (yes versus no) was based on the 24-hour dietary recall data. Habitual physical activity was estimated using the Physical Activity Scale for the Elderly (PASE) questionnaire [33, 34]. The PASE asks for the daily time spent on leisure activities, household activities and occupational activities in the previous week. A higher score (range 0-793) indicates more physical activity. Body height (m) and body weight (kg) were measured using a stadiometer and beam balance, respectively [26]. Body mass index (BMI) was calculated as body weight divided by body height squared (kg/m^2). To minimise the influence of shrinking due to ageing on BMI, body height measured at T1 was used for the calculation of BMI at T1 and T4. Weight change over the 3-year follow-up was calculated as T4 minus T1 (absolute difference). Number of chronic diseases was assessed by the participant's self-report of the presence of each of 20 chronic conditions [35]. Level of pain was assessed using the SF-36 bodily pain index (from the 36-item short form health survey [36]). A higher score (range 0-100) indicates less pain. Depressive symptoms were assessed using the Geriatric Depression Scale (GDS) [37] and cognitive status using the 3MS [38]. All covariates were determined at each annual visit except for sex and education level, which were only assessed at baseline. For descriptive purposes, we expressed daily total protein intake relative to actual body weight (g/kg BW) and adjusted body weight (g/kg aBW). Adjusted body weight is the nearest (ideal) body weight that would place a participant with an undesirable BMI into the healthy range of 18.5 to 25.0 kg/m^2 for adults <71 years or 22.0 to 27.0 kg/m^2 for adults aged ≥71 years [39]. This controls for the deficit or excess in body weight of underweight and overweight people, respectively.

Statistical analyses

Linear regression analyses were performed to examine the associations of the five protein intake parameters with handgrip strength, knee extensor strength and physical performance (TUG) at T4, and their 3-y change. All analyses were *a*

priori stratified by sex because of presumed sex-differences in body composition [17], hormonal milieu [17, 18] and MPS rates [19, 20]. As higher muscle strength values indicate better muscle strength whereas higher TUG values indicate poorer physical performance, in the regression models all TUG values were multiplied by -1 so that positive coefficients (ß) always represent better outcomes.

The regression models included all four protein intake parameters simultaneously. In addition, all regression models were adjusted for the T4 values (or stated otherwise) of age, body height (T1), body weight, habitual physical activity (mean PASE scores of T1 to T4), education level (T1), smoking, alcohol use (mean of T1 to T3), 3-year weight change, cognition, number of medications and pain. As all models were adjusted for factors accounting for energy expenditure (i.e. age, body height, body weight, habitual physical activity) as well as energy balance over time (i.e. 3-year weight change), we did not further adjust for energy intake [40, 41]. When assessing the associations with 3-year change in outcomes, the models were additionally adjusted for the outcome value at T1.

We first examined the associations of four protein intake parameters (daily protein intake, number of protein-providing meals, number of protein-providing snacks and evenness of protein intake distribution across meals) with handgrip strength, knee extensor strength and physical performance at T4. Subsequently, we ran the same model but applied a maximal value of protein dose per meal for calculations of daily protein intake and evenness of protein intake distribution across meals (CV). This procedure was repeated for each potential optimal protein dose. To determine if there is an optimal protein dose per meal for maximum effect size on muscle strength and physical performance, we used the t-values for daily protein intake of each model, under the hypothesis that higher daily protein intake is associated with better muscle strength and physical performance (positive t-value). As such, the dose applied in the model with the highest statistically significant t-value for daily protein intake was considered the optimal protein dose per meal. In this case, results for all protein parameters were presented from this model. If we observed the highest t-value in the model with the actual (non-truncated) values or if t-values did not reach statistical significance in any model, we assumed that no optimal dose actually existed. In that case, results were presented from the model with actual (non-truncated) values. We performed this procedure for each of the three outcomes at T4 and for the 3-year change in each outcome.

Spearman's correlation coefficients were calculated to examine intercorrelations between the four protein intake parameters (i.e. daily protein intake, number of

protein-providing meals, number of protein-providing snacks and evenness of protein intake distribution across meals). Multicollinearity was also checked by the Variation Inflation Factor (VIF). In all models, the VIF for the protein intake parameters was <4 and thus multicollinearity was considered weak [42], allowing the inclusion of all protein parameters in a single model. Normality and linearity were checked by visual inspection of histograms and scatterplots, respectively. Statistical analyses were performed using SPSS Statistics version 26.0 (IBM Corp.). Results were considered statistically significant at P <0.05 (2-sided).

RESULTS

Participant characteristics

Men (n=524) and women (n=574) had a mean (± SD) age of 74.8 ± 4.0 years and 75.2 ± 4.2 years at T1, respectively (**Table 1**). Mean BMI at T1 was 28.1 ± 4.0 kg/m^2 in men and 27.4 ± 4.7 kg/m^2 in women, and was comparable at T4. Number of chronic diseases and medication use increased over the 3-year follow-up, with more than half of the men and women having at least three chronic diseases and using five or more medications at T4.

During the 3-year follow-up, handgrip strength, knee extensor strength and physical performance (TUG) declined, with mean (± SD) 3-year changes of -6.9 ± 9.6 kPa, -4.9 ± 15.8 kg and +0.6 ± 1.8 s in men and -4.9 ± 10.6 kPa, -2.4 ± 10.4 kg and +0.6 ± 2.1 s in women, respectively. Expressed as percentages, the mean (± SD) 3-year changes in the three outcomes were -8.6 ± 13.3%, -4.1 ± 25.1% and +6.9 ± 18.7% in men and -7.0 ± 21.2%, -1.2 ± 31.4% and +7.1 ± 19.3% in women, respectively.

Dietary characteristics

Mean daily protein intake was higher in men (82.7 ± 19.4 g/d) than in women (68.3 ± 15.0 g/d), but was similar relative to adjusted body weight (1.13 ± 0.27 and 1.12 ± 0.26 g/kg aBW/d, respectively) or as percentage of energy (16.1 ± 2.5 and 16.6 ± 2.5%, respectively) (**Table 2**). Also, mean daily protein intake did not differ significantly between T1, T2 and T3 (data not shown), suggesting stable intake over time. The majority of the participants consumed on average three protein-providing meals per day. Snacks contributed for ~5% to daily protein intake. The evenness of protein intake distribution across meals was comparable in men and women. Correlation between each of the protein intake parameters was low: r ≤0.136 in men and r ≤0.192 in women (**Supplemental Table S1**).

Table 1. General characteristics at baseline (T1) and after three years of follow-up (T4) of the community-dwelling older men (*n*=524) and women (*n*=574) from the NuAge cohort

	Men				Women			
	n	T1	*n*	T4	*n*	T1	*n*	T4
Age, years	524	74.8 ± 4.0	524	77.9 ± 4.0	574	75.2 ± 4.2	574	78.3 ± 4.2
Years of education	524	12.0 ± 5.1	-	-	574	11.6 ± 3.9	-	-
Current smoker, *n* (%)	516	33 (6.4)	524	33 (6.3)	562	16 (2.8)	574	18 (3.1)
Consumed alcohol in past 3 years, *n* (%)	-	-	524	416 (79.4)	-	-	574	365 (63.6)
Physical activity (PASE score; 0-793)	524	118 ± 55	491	112 ± 53	573	94 ± 45	544	84 ± 42
Body mass index, kg/m^2	524	28.1 ± 4.0	524	27.9 ± 4.1	574	27.4 ± 4.7	574	27.2 ± 4.8
Weight change over past 3 years, kg	-	-	524	-0.7 ± 3.4	-	-	574	-0.5 ± 3.4
Number of chronic diseases	524		516		574		562	
0		54 (10.3)		44 (8.5)		20 (3.5)		21 (3.7)
1-2		177 (33.8)		163 (31.6)		150 (26.1)		111 (19.8)
≥3		292 (55.9)		309 (59.9)		404 (70.4)		430 (76.5)
Number of medications	524		524		574		574	
0		56 (10.7)		23 (4.4)		30 (5.2)		11 (1.9)
1-4		261 (49.8)		223 (42.6)		252 (43.9)		189 (32.9)
≥5		207 (39.5)		278 (53.1)		292 (50.9)		374 (65.2)
Pain (SF-36 pain index; 0-100)	523	75.9 ± 24.4	524	72.5 ± 25.3	574	67.5 ± 25.3	574	63.9 ± 25.6
Depressive symptoms (GDS score; 0-30)	524	4.3 ± 3.7	507	4.2 ± 4.0	573	5.0 ± 4.3	563	5.0 ± 4.2
Cognitive status (3MS score; 0-100)	523	93.7 ± 4.4	524	91.6 ± 6.1	573	95.0 ± 3.8	574	93.5 ± 5.6
Handgrip strength, kPa	524	78.4 ± 17.6	524	71.5 ± 17.6	574	59.3 ± 16.3	574	54.3 ± 16.0
Knee extensor strength, kg	524	72.8 ± 20.7	524	67.9 ± 19.8	574	43.6 ± 14.0	574	41.2 ± 12.7
Physical performance (Timed Up and Go), s	524	10.0 ± 1.8	524	10.6 ± 2.2	574	10.6 ± 2.1	574	11.2 ± 2.8

All values represent mean ± standard deviation, or stated otherwise. Abbreviations: 3MS, Modified Mini-Mental State Examination; GDS, Geriatric Depression Scale; PASE, Physical Activity Scale for the Elderly; SF-36, 36-item Short form health survey questionnaire.

Protein intake parameters, muscle strength and physical performance in men

Regression models were run in order to identify whether truncating the protein intake per meal to a maximal value would lead to stronger associations between daily protein intake and the outcomes, and thus suggests an optimal protein dose per meal to exist. As expected, with an increase in the maximal value applied, the t-values for daily protein intake increased, reached a maximum (i.e. the optimal dose) and plateaued or slightly decreased (**Figure 1**). For men, optimal protein doses per meal of 30-35 g were observed for knee extensor strength and physical performance at T4 (**Figure 1A**). Applying these thresholds led to statistical

significant associations of higher daily protein intake with better knee extensor strength (ß: 0.218, 95% CI: 0.015, 0.422) and physical performance (ß: 0.029, 95% CI: 0.002, 0.057) at T4 (**Table 3**), while the models based on the actual intakes failed to show associations (**Figures 1A and 1B**). We observed no associations – and thus no optimal protein dose per meal – of daily protein intake with handgrip strength at T4 or with the 3-year change in these outcomes.

The number of protein-providing meals and the number of protein-providing snacks were not associated with any outcome. Evenness of protein intake distribution across meals was not associated with handgrip or knee extensor strength at T4, but a more uneven protein intake distribution across meals was associated with better physical performance at T4 (ß: 1.768, 95% CI: 0.051, 3.484). Also, a more uneven protein intake distribution across meals was associated with less 3-year decline in handgrip strength (ß: 6.670, 95% CI: 1.406, 11.935), but not with the 3-year change in knee extensor strength or physical performance (**Table 3**).

Table 2. Dietary intake characteristics of the community-dwelling older men (*n*=524) and women (*n*=574) from the NuAge cohort

	Men	Women
Daily energy intake, kcal/d	2092 ± 445	1674 ± 337
Daily protein intake, g/d	82.7 ± 19.4	68.3 ± 15.0
Daily protein intake as a percentage of energy (E%)	16.1 ± 2.5	16.6 ± 2.5
Daily protein intake, g/kg BW/d	1.06 ± 0.28	1.07 ± 0.30
Daily protein intake, g/kg aBW/d	1.13 ± 0.27	1.12 ± 0.26
Protein intake from main meals, g/d	78.7 ± 18.9	64.7 ± 14.6
Protein intake from snacks, g/d	2.6 (1.1-5.4) [a]	2.6 (1.2-4.9) [a]
Mean number of protein-providing meals, *n* (%)		
<2.86 meals/d	82 (15.6%)	64 (11.1%)
2.86-2.99 meals/d	85 (16.2%)	88 (15.3%)
3.00 meals/d	357 (68.1%)	422 (73.5%)
Mean number of protein-providing snacks, *n* (%)		
Tertile 1 (≤0.67 snacks/d)	188 (35.9%)	173 (30.1%)
Tertile 2 (≥0.78 and ≤1.22 snacks/d)	169 (32.3%)	204 (35.5%)
Tertile 3 (≥1.33 snacks/d)	167 (31.9%)	197 (34.3%)
Evenness of protein intake distribution across meals (CV)	0.59 ± 0.15	0.60 ± 0.14

All values represent mean ± standard deviation, or stated otherwise, and are calculated as average over the three years (nine recall days). [a] Median (interquartile range). Abbreviations: aBW, adjusted body weight (the nearest ideal body weight that would put a participant with an undesirable body mass index into the healthy range of 18.5 to 25.0 kg/m² for adults <71 years or 22.0 to 27.0 kg/m² for adults aged ≥71 years); BW, (actual) body weight; CV, coefficient of variation.

Protein intake parameters, muscle strength and physical performance in women

In women, optimal protein doses per meal of 35-50 g were observed for knee extensor strength and physical performance at T4 and for 3-year change in physical performance (**Figures 1C and 1D**). Applying these thresholds led to associations of a higher daily protein intake with better knee extensor strength (ß: 0.103, 95% CI: 0.003, 0.203) and physical performance (ß: 0.017, 95% CI: 0.000, 0.034), but not with handgrip strength, at T4 (**Table 4**). Higher daily protein intake was also associated with less 3-year decline in physical performance (ß: 0.019, 95% CI: 0.000, 0.039), but not with the 3-year change in handgrip or knee extensor strength.

A higher number of protein-providing snacks was associated with higher handgrip strength (tertile 2 versus 1, ß: 4.817, 95% CI: 1.732, 7.902; tertile 3 versus 1, ß: 4.103, 95% CI: 0.965, 7.240) and knee extensor strength (tertile 2 versus 1, ß: 3.045, 95% CI: 0.639, 5.451; tertile 3 versus 1, ß: 2.120, 95% CI: -0.323, 4.563) at T4, and with the 3-year change in handgrip strength (tertile 2 versus 1, ß: 2.091, 95% CI: 0.079, 4.103; tertile 3 versus 1, ß: 1.113, 95% CI: -0.934, 3.161). Number of protein-providing snacks was not associated with physical performance. Also, no associations were observed of number of protein-providing meals or evenness of protein intake distribution across meals with any outcome (**Table 4**).

DISCUSSION

The present study is the first to examine prospective associations of different protein intake parameters simultaneously with muscle strength and physical performance in community-dwelling older men and women. Higher daily protein intake was associated with higher knee extensor strength and better physical performance (TUG) in both sexes and with less 3-year decline in physical performance in women. We observed optimal protein doses per meal of ~30-35 g in men and ~35-50 g in women for these outcomes. Daily protein intake was not associated with handgrip strength. In men, more uneven protein intake distribution across meals was associated with better physical performance and with less decline in handgrip strength. In women, higher number of protein-providing snacks was associated with higher handgrip strength and knee extensor strength and with less decline in handgrip strength. In neither sex was the number of protein-providing meals associated with outcomes.

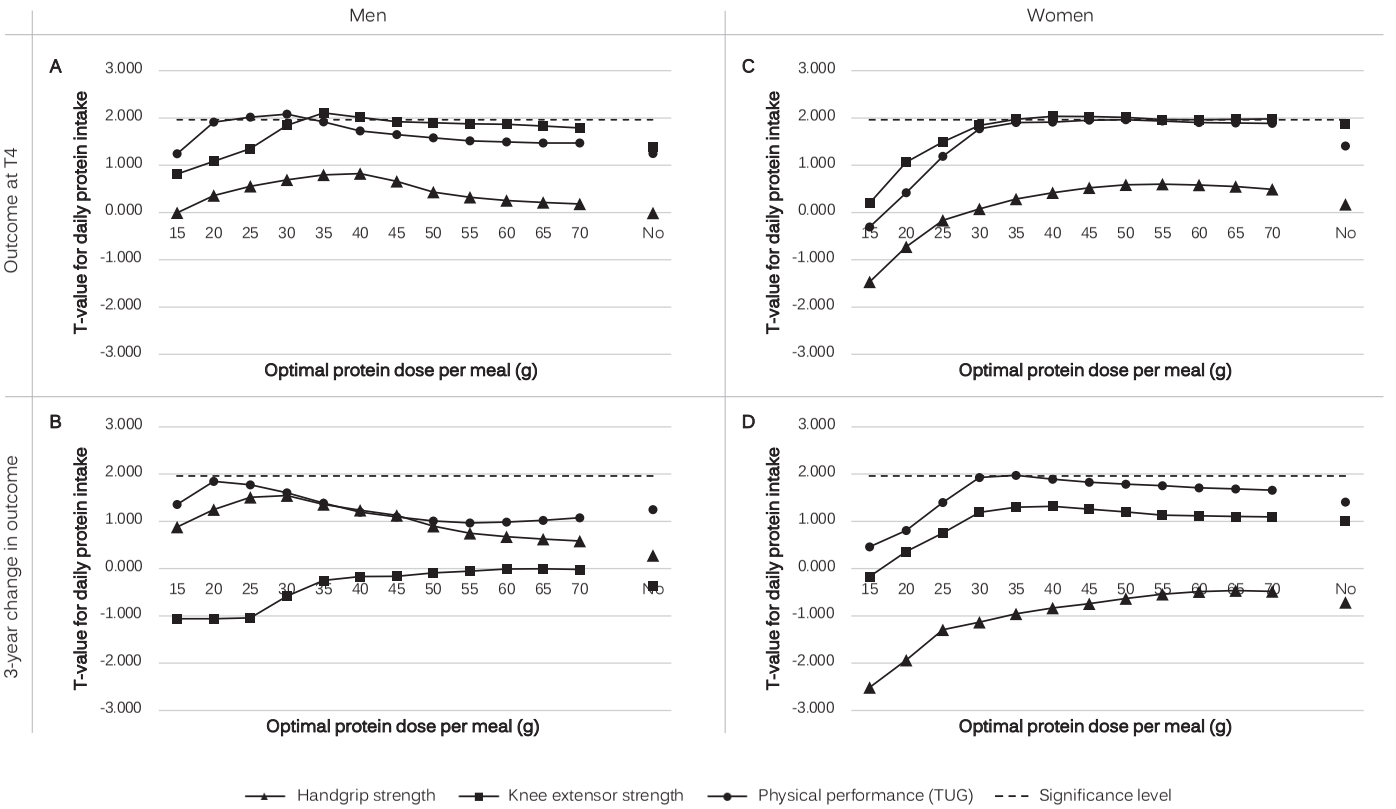


Figure 1. T-values for the associations of daily protein intake with handgrip strength (▲), knee extensor strength (■) and physical performance (•) at T4 and with the 3-year changes in these outcomes in community-dwelling older men (*n*=524; A and B) and women (*n*=574; C and D) from the NuAge cohort. T-values for physical performance were multiplied by -1 to facilitate comparison of the t-values for the different outcomes. The dashed line represents the t-value for statistical significance (t=1.960). Abbreviations: TUG, Timed Up and Go.

Table 3. Associations of protein intake parameters with handgrip strength, knee extensor strength and physical performance at T4, and with their 3-year change in community-dwelling older men (*n*=524) from the NuAge study

	Handgrip strength (kPa)		Knee extensor strength (kg)		Physical performance (TUG (s))[a]	
	ß (95% CI)	*P-value*	*ß (95% CI)*	*P-value*	*ß (95% CI)*	*P-value*
Outcome at T4[b]	Optimal dose per meal[c]: no		Optimal dose per meal[c]: 35 g		Optimal dose per meal[c]: 30 g	
Daily protein intake (g)	0.000 (-0.074, 0.073)	0.993	0.218 (0.015, 0.422)	0.035	0.029 (0.002, 0.057)	0.038
Mean number of protein-providing meals: 2.86-2.99 versus <2.86 meals/d[d]	-1.434 (-6.198, 3.331)	0.555	-2.310 (-7.985, 3.365)	0.424	-0.599 (-1.231, 0.032)	0.063
Mean number of protein-providing meals: 3.00 versus <2.86 meals/d[d]	-0.378 (-4.147, 3.390)	0.844	-4.169 (-8.764, 0.425)	0.075	-0.357 (-0.872, 0.159)	0.175
Mean number of protein-providing snacks: tertile 2 versus tertile 1[e]	-0.586 (-3.842, 2.670)	0.724	0.384 (-3.450, 4.217)	0.844	-0.109 (-0.531, 0.313)	0.612
Mean number of protein-providing snacks: tertile 3 versus tertile 1[e]	-1.140 (-4.464, 2.184)	0.501	-0.393 (-4.393, 3.608)	0.847	-0.173 (-0.622, 0.275)	0.448
Evenness of protein intake distribution across meals (CV)	7.009 (-2.035, 16.053)	0.128	11.427 (-3.156, 26.011)	0.124	1.768 (0.051, 3.484)	0.044
3-year change in outcome[b,f]	Optimal dose per meal[c]: no		Optimal dose per meal[c]: no		Optimal dose per meal[c]: no	
Daily protein intake (g)	0.006 (-0.037, 0.049)	0.781	-0.013 (-0.080, 0.055)	0.712	0.005 (-0.003, 0.013)	0.212
Mean number of protein-providing meals: 2.86-2.99 versus <2.86 meals/d[d]	-0.897 (-3.670, 1.877)	0.526	-1.976 (-6.321, 2.368)	0.372	-0.174 (-0.697, 0.349)	0.513
Mean number of protein-providing meals: 3.00 versus <2.86 meals/d[d]	-1.106 (-3.300, 1.088)	0.322	-1.641 (-5.079, 1.797)	0.349	-0.107 (-0.520, 0.306)	0.611
Mean number of protein-providing snacks: tertile 2 versus tertile 1[e]	1.574 (-0.326, 3.474)	0.104	0.575 (-2.393, 3.543)	0.704	-0.044 (-0.401, 0.312)	0.807
Mean number of protein-providing snacks: tertile 3 versus tertile 1[e]	1.694 (-0.249, 3.637)	0.087	-0.067 (-3.097, 2.964)	0.965	-0.050 (-0.414, 0.315)	0.790
Evenness of protein intake distribution across meals (CV)	6.670 (1.406, 11.935)	0.013	4.545 (-3.699, 12.790)	0.279	0.372 (-0.621, 1.365)	0.462

[a] All TUG values were multiplied by -1 so that positive coefficients (ß) always represent better outcomes. [b] Results are from the fully-adjusted regression models, including age, body height, body weight, physical activity, education level, smoking, alcohol use, weight change, cognition, number of medications and pain. [c] Results are from the regression analyses with the optimal dose of protein per meal as specified. [d] Number of protein-providing meals was categorised based on the mean number of meals/d over the nine recall days. [e] Number of protein-providing snacks was categorised based on the mean number of snacks/d over the nine recall days into tertiles: tertile 1 (≤0.67 snacks/d), tertile 2 (≥0.78 and ≤1.22 snacks/d) and tertile 3 (≥1.33 snacks/d). [f] Additionally adjusted for outcome value at T1. Abbreviations: CI, confidence interval; CV, coefficient of variation; TUG, Timed Up and Go.

Table 4. Associations of protein intake parameters with handgrip strength, knee extensor strength and physical performance at T4, and with their 3-year change in community-dwelling older women (*n*=574) from the NuAge study

	Handgrip strength (kPa)		Knee extensor strength (kg)		Physical performance (TUG (s))[a]	
	ß (95% CI)	*P-value*	*ß (95% CI)*	*P-value*	*ß (95% CI)*	*P-value*
Outcome at T4[b]	Optimal dose per meal[c]: no		Optimal dose per meal[c]: 40 g		Optimal dose per meal[c]: 50 g	
Daily protein intake (g)	0.008 (-0.081, 0.097)	0.863	0.103 (0.003, 0.203)	0.043	0.017 (0.000, 0.034)	0.050
Mean number of protein-providing meals: 2.86-2.99 versus <2.86 meals/d[d]	3.404 (-1.523, 8.331)	0.175	0.946 (-2.896, 4.787)	0.629	0.515 (-0.268, 1.299)	0.197
Mean number of protein-providing meals: 3.00 versus <2.86 meals/d[d]	0.251 (-3.887, 4.390)	0.905	0.190 (-3.055, 3.435)	0.908	-0.031 (-0.691, 0.629)	0.927
Mean number of protein-providing snacks: tertile 2 versus tertile 1[e]	4.817 (1.732, 7.902)	0.002	3.045 (0.639, 5.451)	0.013	-0.027 (-0.517, 0.464)	0.915
Mean number of protein-providing snacks: tertile 3 versus tertile 1[e]	4.103 (0.965, 7.240)	0.010	2.120 (-0.323, 4.563)	0.089	0.098 (-0.401, 0.596)	0.701
Evenness of protein intake distribution across meals (CV)	-1.792 (-10.876, 7.292)	0.698	-2.049 (-10.405, 6.307)	0.630	0.698 (-0.877, 2.273)	0.384
3-year change in outcome[b,f]	Optimal dose per meal[c]: no		Optimal dose per meal[c]: no		Optimal dose per meal[c]: 35 g	
Daily protein intake (g)	-0.021 (-0.079, 0.037)	0.476	0.027 (-0.026, 0.079)	0.317	0.019 (0.000, 0.039)	0.049
Mean number of protein-providing meals: 2.86-2.99 versus <2.86 meals/d[d]	1.069 (-2.134, 4.271)	0.512	1.333 (-1.566, 4.232)	0.367	0.100 (-0.541, 0.740)	0.760
Mean number of protein-providing meals: 3.00 versus <2.86 meals/d[d]	-1.349 (-4.037, 1.340)	0.325	1.034 (-1.401, 3.470)	0.404	-0.292 (-0.834, 0.249)	0.289
Mean number of protein-providing snacks: tertile 2 versus tertile 1[e]	2.091 (0.079, 4.103)	0.042	1.033 (-0.792, 2.859)	0.267	-0.205 (-0.605, 0.196)	0.316
Mean number of protein-providing snacks: tertile 3 versus tertile 1[e]	1.113 (-0.934, 3.161)	0.286	1.403 (-0.444, 3.251)	0.136	-0.018 (-0.425, 0.388)	0.929
Evenness of protein intake distribution across meals (CV)	-0.950 (-6.847, 4.946)	0.752	-4.651 (-9.995, 0.693)	0.088	0.779 (-0.698, 2.257)	0.301

[a] All TUG values were multiplied by -1 so that positive coefficients (ß) always represent better outcomes. [b] Results are from the fully-adjusted regression models, including age, body height, body weight, physical activity, education level, smoking, alcohol use, weight change, cognition, number of medications and pain. [c] Results are from the regression analyses with the optimal dose of protein per meal as specified. [d] Number of protein-providing meals was categorised based on the mean number of meals/d over the nine recall days. [e] Number of protein-providing snacks was categorised based on the mean number of snacks/d over the nine recall days into tertiles: tertile 1 (≤0.67 snacks/d), tertile 2 (≥0.78 and ≤1.22 snacks/d) and tertile 3 (≥1.33 snacks/d). [f] Additionally adjusted for outcome value at T1. Abbreviations: CI, confidence interval; CV, coefficient of variation; TUG, Timed Up and Go.

Higher daily protein intake was associated with higher knee extensor strength and physical performance (TUG) at T4 in both sexes. Although these findings confirm our hypothesis, very few studies examined protein intake in relation to these outcomes specifically. Our results are nonetheless similar to the findings of Farsijani et al. [25], also from the NuAge data, but contrast with the (cross-sectional) null findings of Granic et al. [23]. This discrepancy may be explained by differences in age, follow-up time, dietary assessment and adjustment for different confounders, but also by the fact that Granic et al. did not account for the potential ceiling effect of protein intake per meal. Regression coefficients were indeed severely attenuated in the present study when no maximal value of protein per meal was applied (**Figure 1**). Surely, more prospective research is needed to replicate our findings. Our observed associations for daily protein intake were not statistically significant when analysed in relation to the 3-year change in outcomes, except physical performance in women. The decline in muscle strength and physical performance in our analytic sample (1-9%) may have been too small over the limited follow-up to observe associations. Moreover, participants included in the present study had relatively high habitual daily protein intake compared to the current recommended dietary allowance. The potential contribution of protein to muscle strength and physical performance may then have already been integrated within the outcome measurements at baseline. Also, we did not measure change in protein intake over time, but it would unlikely have explained the 3-year changes in outcomes since our data showed that daily protein intake remained stable over years. The decrease in muscle strength and physical performance in the NuAge cohort may likely be related to the ageing process per se and other factors (e.g. sedentary behaviour) than changes in dietary protein.

The observed optimal protein doses per meal of 30-35 g in men and 35-50 g in women were comparable with findings from metabolic dose-response studies [43-45]. For example, Cuthbertson et al. [43] observed a significant increase in MPS up to intakes of 10 g essential amino acids (~20 g whey protein or ~30 g protein in a regular meal) in older men (mean age: 70 ± 6 years) at rest, but no significant additional effect for higher intakes. Similarly, Yang et al. [45] found the optimal dose of whey protein for maximal MPS in older men (mean age: 71 ± 4 years) to be 20 g at rest and 40 g in combination with leg-based resistance exercise. Only one (cross-sectional) observational study addressed this protein intake parameter and showed that more frequent consumption of meals was most strongly associated with higher muscle strength when protein doses were 30-45 g/meal [30], which is also consistent with our results. The higher optimal protein dose per meal observed in women than in men may be due to the reduced sensitivity (i.e. lower MPS response

to anabolic stimuli) reported in older women [18, 20]. Hence, women might need higher protein doses to reach optimal MPS than men. Moreover, women in the NuAge cohort appear on average less physically active than men whereas physical activity was reported to potentially overcome anabolic resistance in older adults [46]. Therefore, while physical activity might further increase MPS rates reached by protein provision only [45, 46], it may also decrease the need for high protein intake to preserve optimal muscle and physical function in ageing.

Number of protein-providing meals was not associated with any outcome in both sexes. This might be the result of the observation that most NuAge participants had three meals a day most (if not all) of the time, making it difficult to reveal any association. Though, number of protein-providing snacks was associated with (change in) muscle strength in women, independently of daily protein intake. Snacking may provide more anabolic opportunities during the postprandial state when MPS reduces and muscle protein breakdown elevates. Interestingly, in our analytic sample, women consumed more protein-providing snacks than men, which may explain our results. Women also had a smaller protein dose per meal, which may make the role of snacking more important than in men. No previous studies examined the effect of snacking in older adults, so future research is needed to explore this hypothesis.

In the present study, we did not observe that a more even distribution of protein across meals was associated with better muscle strength or physical performance, as some authors have postulated [6, 10, 47]. On the contrary, we observed either no association or that a more uneven protein intake distribution was better in terms of muscle strength and physical performance (only in men). Our results are in contrast with those reported by Farsijani et al. [25] and Granic et al. [23], who did not observe an association between evenness of protein intake distribution and physical performance. As for daily protein intake, these discrepancies might be partly explained by the optimal protein dose per meal we took into account in calculations of the CV. Also, daily protein intake is slightly negatively correlated with the CV. As such, the CV may have captured the effect of daily protein intake in these studies, leading to results suggesting beneficial effects of even distribution. The sex difference observed in our study may be explained by women being probably less likely to reach the optimal protein dose per meal as often as men given their lower daily protein intake and higher optimal protein dose per meal. More research focused on protein intake distribution – independent of total protein intake and number of meals – in relation to function outcomes is required.

The main strength of the present study is the in-depth and unique approach to study protein intake, i.e. considering its multiple facets. Moreover, dietary intake was assessed using nine 24-hour recalls collected over three years, which provided representative estimates of habitual dietary habits. Indeed, all significant coefficients were severely attenuated when calculations of the protein intake parameters were estimated based on only three recalls (one from each T1, T2 and T3; data not shown). Other strengths include the prospective study design and large number of potent confounders that we adjusted for. Some limitations must be discussed as well. First, this cohort comprised predominantly Caucasians, so caution is required when applying these results to other ethnicities. Second, other protein intake parameters may be related to muscle strength and physical performance, such as the protein quality [48, 49]. Last, due to the observational nature of this study, the causal relationship remains uncertain and any residual confounding (e.g. by fat mass) cannot be dismissed.

To conclude, in this cohort of community-dwelling older adults being generally well-functioning at baseline, higher daily protein intake over three years, up to a maximal per-meal dose of 30-35 g in men and 35-50 g in women, was associated with better knee extensor strength and physical performance, but not handgrip strength. The role of protein intake distribution across meals or the number of protein-providing meals or snacks remains uncertain and could depend on the protein dose per meal. Nevertheless, our results imply that more aspects of protein intake may contribute to muscle strength and physical performance than solely the daily protein quantity, notably the protein dose per meal. Our results also support the idea that large quantities of protein in one meal (>50 g) might not provide further benefits for muscle and physical function. Future prospective research should examine the role and interplay of different protein intake parameters in maintenance of function outcomes in older adults to further refine protein recommendations.

REFERENCES

1. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci. 2006;61(10):1059-1064.
2. Stevens PJ, Syddall HE, Patel HP, Martin HJ, Cooper C, Aihie Sayer A. Is grip strength a good marker of physical performance among community-dwelling older people? J Nutr Health Aging. 2012;16(9):769-774.
3. Deer RR, Volpi E. Protein intake and muscle function in older adults. Curr Opin Clin Nutr Metab Care. 2015;18(3):248-253.
4. Strasser B, Volaklis K, Fuchs D, Burtscher M. Role of Dietary Protein and Muscular Fitness on Longevity and Aging. Aging Dis. 2018;9(1):119-132.
5. Landi F, Calvani R, Tosato M, Martone AM, Ortolani E, Savera G, et al. Protein Intake and Muscle Health in Old Age: From Biological Plausibility to Clinical Evidence. Nutrients. 2016;8(5).
6. Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. J Am Med Dir Assoc. 2013;14(8):542-559.
7. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, D.C.; 2005.
8. Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. Clin Nutr. 2014;33(6):929-936.
9. Deutz NE, Wolfe RR. Is there a maximal anabolic response to protein intake with a meal? Clin Nutr. 2013;32(2):309-313.
10. Paddon-Jones D, Campbell WW, Jacques PF, Kritchevsky SB, Moore LL, Rodriguez NR, et al. Protein and healthy aging. Am J Clin Nutr. 2015;101(6):1339S-1345S.
11. Witard OC, Wardle SL, Macnaughton LS, Hodgson AB, Tipton KD. Protein Considerations for Optimising Skeletal Muscle Mass in Healthy Young and Older Adults. Nutrients. 2016;8(4):181.
12. Mishra S, Goldman JD, Sahyoun NR, Moshfegh AJ. Association between dietary protein intake and grip strength among adults aged 51 years and over: What We Eat in America, National Health and Nutrition Examination Survey 2011-2014. PLoS One. 2018;13(1):e0191368.
13. Berryman CE, Lieberman HR, Fulgoni VL, 3rd, Pasiakos SM. Protein intake trends and conformity with the Dietary Reference Intakes in the United States: analysis of the National Health and Nutrition Examination Survey, 2001-2014. Am J Clin Nutr. 2018;108(2):405-413.
14. O'Leary F, Grech A, Sui Z, Cheng H, Rangan A, Hirani V. Older Australians are eating more protein: Secondary analysis of the 1995 & 2011/12 national nutrition surveys. Eur J Clin Nutr. 2019.
15. Farsijani S, Morais JA, Payette H, Gaudreau P, Shatenstein B, Gray-Donald K, et al. Relation between mealtime distribution of protein intake and lean mass loss in free-living older adults of the NuAge study. Am J Clin Nutr. 2016;104(3):694-703.
16. Rippin HL, Hutchinson J, Jewell J, Breda JJ, Cade JE. Adult Nutrient Intakes from Current National Dietary Surveys of European Populations. Nutrients. 2017;9(12).
17. Anderson LJ, Liu H, Garcia JM. Sex Differences in Muscle Wasting. Adv Exp Med Biol. 2017;1043:153-197.
18. Hansen M, Kjaer M. Influence of sex and estrogen on musculotendinous protein turnover at rest and after exercise. Exerc Sport Sci Rev. 2014;42(4):183-192.
19. Henderson GC, Dhatariya K, Ford GC, Klaus KA, Basu R, Rizza RA, et al. Higher muscle protein synthesis in women than men across the lifespan, and failure of androgen administration to amend age-related decrements. FASEB J. 2009;23(2):631-641.
20. Smith GI, Atherton P, Villareal DT, Frimel TN, Rankin D, Rennie MJ, et al. Differences in muscle protein synthesis and anabolic signaling in the postabsorptive state and in response to food in 65-80 year old men and women. PLoS One. 2008;3(3):e1875.

21. McLean RR, Mangano KM, Hannan MT, Kiel DP, Sahni S. Dietary Protein Intake Is Protective Against Loss of Grip Strength Among Older Adults in the Framingham Offspring Cohort. J Gerontol A Biol Sci Med Sci. 2016;71(3):356-361.
22. Beasley JM, Wertheim BC, LaCroix AZ, Prentice RL, Neuhouser ML, Tinker LF, et al. Biomarker-calibrated protein intake and physical function in the Women's Health Initiative. J Am Geriatr Soc. 2013;61(11):1863-1871.
23. Granic A, Mendonca N, Sayer AA, Hill TR, Davies K, Adamson A, et al. Low protein intake, muscle strength and physical performance in the very old: The Newcastle 85+ Study. Clin Nutr. 2017.
24. Bartali B, Frongillo EA, Stipanuk MH, Bandinelli S, Salvini S, Palli D, et al. Protein intake and muscle strength in older persons: does inflammation matter? J Am Geriatr Soc. 2012;60(3):480-484.
25. Farsijani S, Payette H, Morais JA, Shatenstein B, Gaudreau P, Chevalier S. Even mealtime distribution of protein intake is associated with greater muscle strength, but not with 3-y physical function decline, in free-living older adults: the Quebec longitudinal study on Nutrition as a Determinant of Successful Aging (NuAge study). Am J Clin Nutr. 2017;106(1):113-124.
26. Gaudreau P, Morais JA, Shatenstein B, Gray-Donald K, Khalil A, Dionne I, et al. Nutrition as a determinant of successful aging: description of the Quebec longitudinal study Nuage and results from cross-sectional pilot studies. Rejuvenation Res. 2007;10(3):377-386.
27. Desrosiers J, Rochette A, Payette H, Grégoire L, Boutier V, Lazowski DA. Upper extremity strength measurements with the belt-resisted method: Test-retest and interrater reliability studies. Canadian Journal of Rehabilitation. 1998;11:149-155.
28. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc. 1991;39(2):142-148.
29. Conway JM, Ingwersen LA, Vinyard BT, Moshfegh AJ. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. Am J Clin Nutr. 2003;77(5):1171-1178.
30. Loenneke JP, Loprinzi PD, Murphy CH, Phillips SM. Per meal dose and frequency of protein consumption is associated with lean mass and muscle performance. Clin Nutr. 2016;35(6):1506-1511.
31. Kim IY, Deutz NEP, Wolfe RR. Update on maximal anabolic response to dietary protein. Clin Nutr. 2018;37(2):411-418.
32. Phillips SM, Chevalier S, Leidy HJ. Protein "requirements" beyond the RDA: implications for optimizing health. Appl Physiol Nutr Metab. 2016;41(5):565-572.
33. Washburn RA, McAuley E, Katula J, Mihalko SL, Boileau RA. The physical activity scale for the elderly (PASE): evidence for validity. J Clin Epidemiol. 1999;52(7):643-651.
34. Washburn RA, Smith KW, Jette AM, Janney CA. The Physical Activity Scale for the Elderly (PASE): development and evaluation. J Clin Epidemiol. 1993;46(2):153-162.
35. Bouchard DR, Dionne IJ, Brochu M. Sarcopenic/obesity and physical capacity in older men and women: data from the Nutrition as a Determinant of Successful Aging (NuAge)-the Quebec longitudinal Study. Obesity (Silver Spring). 2009;17(11):2082-2088.
36. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30(6):473-483.
37. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, et al. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res. 1982;17(1):37-49.
38. Teng EL, Chui HC. The Modified Mini-Mental State (3MS) examination. J Clin Psychiatry. 1987;48(8):314-318.
39. Berner LA, Becker G, Wise M, Doi J. Characterization of dietary protein among older adults in the United States: amount, animal sources, and meal patterns. J Acad Nutr Diet. 2013;113(6):809-815.
40. Jakes RW, Day NE, Luben R, Welch A, Bingham S, Mitchell J, et al. Adjusting for energy intake--what measure to use in nutritional epidemiological studies? Int J Epidemiol. 2004;33(6):1382-1386.
41. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997;65(4 Suppl):1220S-1228S; discussion 1229S-1231S.

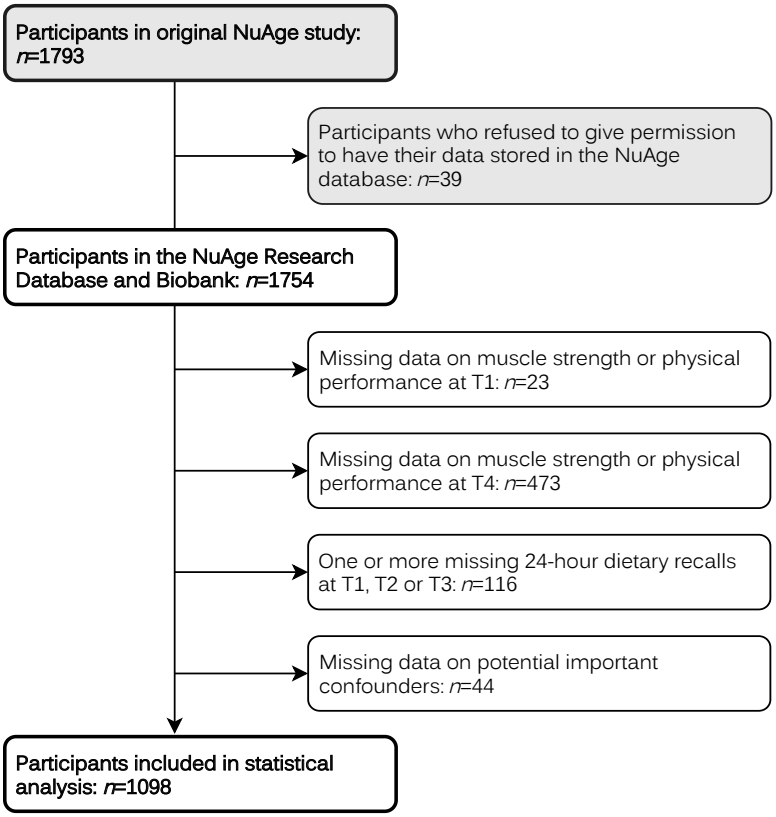
42. O'Brien RM. A caution regarding rules of thumb for variance inflation factors. Qual Quant. 2007;41(5):673-690.
43. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J. 2005;19(3):422-424.
44. Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD, et al. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. J Gerontol A Biol Sci Med Sci. 2015;70(1):57-62.
45. Yang Y, Breen L, Burd NA, Hector AJ, Churchward-Venne TA, Josse AR, et al. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. Br J Nutr. 2012;108(10):1780-1788.
46. Shad BJ, Thompson JL, Breen L. Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. Am J Physiol Endocrinol Metab. 2016;311(5):E803-E817.
47. Witard OC, McGlory C, Hamilton DL, Phillips SM. Growing older with health and vitality: a nexus of physical activity, exercise and nutrition. Biogerontology. 2016;17(3):529-546.
48. Casperson SL, Sheffield-Moore M, Hewlings SJ, Paddon-Jones D. Leucine supplementation chronically improves muscle protein synthesis in older adults consuming the RDA for protein. Clin Nutr. 2012;31(4):512-519.
49. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr. 2003;78(2):250-258.

SUPPLEMENTAL MATERIAL

Supplemental Table S1. Spearman's correlation coefficients between the four protein intake parameters in community-dwelling older men (*n*=524) and women (*n*=574) from the NuAge cohort

	Men				Women			
	Daily protein intake (g/d)	Mean number of protein-providing meals	Mean number of protein-providing snacks	Evenness of protein intake distribution across meals (CV)	Daily protein intake (g/d)	Mean number of protein-providing meals	Mean number of protein-providing snacks	Evenness of protein intake distribution across meals (CV)
Daily protein intake (g/d)	1.000	0.102*	0.022	-0.136*	1.000	0.154*	0.051	-0.192*
Mean number of protein-providing meals		1.000	-0.018	-0.006		1.000	0.022	-0.183*
Mean number of protein-providing snacks			1.000	-0.023			1.000	-0.108*
Evenness of protein intake distribution across meals (CV)				1.000				1.000

Abbreviations: CV, coefficient of variation. * P <0.05 (2-sided)



Supplemental Figure S1. Flow chart of NuAge participants included in the statistical analyses. Abbreviations: T1, baseline; T4, 3-year follow-up.